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Silicon Deprivation Decreases Collagen Formation in Wounds and Bone, and Ornithine Transaminase Enzyme Activity in Liver

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ABSTRACT

We have shown that silicon (Si) deprivation decreases the collagen concentration in bone of 9-wk-old rats. Finding that Si deprivation also affects collagen at different stages in bone development, collagen-forming enzymes, or collagen deposition in other tissues would have implications that Si is important for both wound healing and bone formation. Therefore, 42 rats in experiment 1 and 24 rats in experiment 2 were fed a basal diet containing 2 or 2.6 µg Si/g, respectively, based on ground corn and casein, and supplemented with either 0 or 10 µg Si/g as sodium metasilicate. At 3 wk, the femur was removed from 18 of the 42 rats in experiment 1 for hydroxyproline analysis. A polyvinyl sponge was implanted beneath the skin of the upper back of each of the 24 remaining rats. Sixteen hours before termination and 2 wk after the sponge had been implanted, each rat was given an oral dose of ^{14}C-proline (1.8 µCi/100 g body wt). The total amount of hydroxyproline was significantly lower in the tibia and sponges taken from Si-deficient animals than Si-supplemented rats. The disintegrations per minute of ^{14}C-proline were significantly higher in sponge extracts from Si-

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deficient rats than Si-supplemented rats. Additional evidence of aberrations in proline metabolism with Si deprivation was that liver ornithine aminotransferase was significantly decreased in Si-deprived animals in experiment 2. Findings of an increased accumulation of ^{14}C-proline and decreased total hydroxyproline in implanted sponges and decreased activity of a key enzyme in proline synthesis (liver ornithine aminotransferase) in Si-deprived animals indicates an aberration in the formation of collagen from proline in sites other than bone that is corrected by Si. This suggests that Si is a nutrient of concern in wound healing as well as bone formation.

Index Entries: Silicon; collagen; wound healing; bone; ornithine transaminase.

INTRODUCTION

Signs of silicon (Si) deficiency were described for chickens and rats *(1–3)*, which led to the suggestion that silicon is an essential element *(4)*. Most of the reported signs of silicon deficiency indicated an aberrant metabolism of connective tissue and bone. Abnormalities in articular cartilage and connective tissue were produced in chicks fed silicon-deficient diets *(5)*. Subsequent experiments demonstrated that silicon-deficient chicks had structural abnormalities and depressed collagen content of the frontal bones, long-bone abnormalities characterized by small, poorly formed joints, and defective endochondral bone growth *(6)*. These findings led Carlisle *(7)* to question, as early as 1975, whether silicon deficiency might also impact wound healing.

Two experiments were conducted to ascertain the effect of silicon deprivation on collagen variables in addition to the collagen content in bone. In experiment 1, the effect of adequate or inadequate dietary silicon on wound healing was assessed by the incorporation of proline and accumulation of collagen in sponges surgically implanted in rats. A secondary objective was to assess, through hydroxyproline determination, the effect of dietary silicon on deposition of collagen in bones early in the bone formation process. For experiment 2, the objective was to determine whether inadequate silicon would alter the activity of ornithine aminotransferase, an enzyme needed to form proline, which is a substrate for collagen formation. Blood variables and bone mineral composition were also assessed to substantiate the achievement of a silicon deprivation.

MATERIALS AND METHODS

In experiment 1, 42, and in experiment 2, 24, weanling male Sprague–Dawley (Sasco, Omaha, NE) rats, age 21 d, were weighed upon arrival and housed 3 per all plastic cage measuring 50 × 24 × 16 cm and located inside a laminar airflow rack (Lab Products, Maywood NJ) *(8)*. In

both experiments, rats were randomly assigned to one of two treatment groups, with no significant differences in weight (mean of 39 g). The independent variables were, per gram fresh diet, silicon (as sodium metasilicate) at 0 (–Si) or 10 µg (+Si). The silicon supplement was reagent grade (J. T. Baker, Phillipsburg, NJ).

The composition of the basal diet has been reported *(9)*. Analysis indicated that the basal diets contained about 2.0 and 2.6 µg Si/g in experiments 1 and 2, respectively. Fresh food in plastic cups was provided ad libitum each day. The diets were mixed 3 d before the start of the experiment. The diets were not pelleted and were stored at –16°C in tightly capped plastic containers. The rats were fed deionized water (Super Q System, Millipore Corp., Bedford, MA) in plastic cups. Absorbent paper under the false-bottom cages was changed daily. Room temperature was maintained at 23°C. Room lighting was controlled automatically to provide 12 h each of light and dark. Animals were weighed and provided clean cages weekly.

At wk 3 and following a 16-h fast, 18 (9 from –Si and 9 from +Si groups) of the 42 rats in experiment 1 were weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. After removal, the right tibia was frozen and later analyzed for hydroxyproline to assess bone collagen content. After thawing, the tibia was cleaned of adhering tissue, dried in a freeze-dryer, and hydrolyzed in 6 *N* HCL (1 : 5 w/v) at 105°C. The hydrolysate was analyzed for hydroxyproline by the method of Podenphant et al. *(10)*. Hydroxyproline was expressed per gram dry weight of tibia.

A 0.12- to 0.15-g polyvinyl sponge (M-PACT, Eudora Kansas) was autoclaved, softened in sterile saline, and then surgically implanted lengthwise under ether anesthesia between the shoulder blades and beneath the skin of the upper back of each of the 24 remaining rats in experiment 1. The incision was closed with surgical staples. Sixteen hours before termination, and 2 wk after the sponge had been implanted, each rat was injected intraperitoneally with a dose of ^{14}C-proline (1.8 µCi/100 g body wt). Following a 16-h fast, animals were weighed and decapitated subsequent to sodium pentobarbital anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. The sponge was removed, weighed to obtain wet weight, dried, and then weighed again to obtain dry tissue weight; then, collagen was extracted with a pepsin–acetic acid solution, followed by autoclaving. Aliquots of the extracted collagen were placed in glass vials containing 1 *N* NaOH and heated followed by the addition of 30% hydrogen peroxide to decolorize. Scintillation fluid was added for the determination of the disintegrations per minute (DPMs) by liquid scintillation (Packard Instrument Co., Meriden CT). DPMs were expressed per gram dry tissue in the sponge. Additional triplicate aliquots were hydrolyzed by 6 *N* HCl and then analyzed for hydroxyproline concentrations by the procedure outlined earlier. Hydroxyproline was expressed per gram dry tissue.

The 24 rats in experiment 2 were fed their diets for 7 wk, then weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. Microhematocrits were determined on the heparinized heart blood. Hemoglobin was determined by using a cyanmethemoglobin method (Sigma Chemical Co., St. Louis MO). Red blood cells, white blood cells, and platelets were counted and the percentage of neutrophils, lymphocytes, eosinophils, basophils, and monocytes were determined by using a hematology analyzer (Cell-Dyn 3500, Abbott Park, Chicago IL). The femur was removed and frozen for mineral analysis.

The liver was removed and weighed, and a portion was frozen in liquid nitrogen and stored for 24 h in a –70°C freezer, before determining ornithine transaminase activity. To determine ornithine transaminase activity, approximately 0.5 g of liver were homogenized with a Teflon pestle in 2.0 mL (4 volumes) of 10% sucrose to make a 20% tissue homogenate. The tissue homogenate was frozen overnight in –20°C freezer, then thawed, sonicated, and centrifuged. A 0.1-mL aliquot of supernatant was added to a reaction mixture brought to 37°C and containing 0.167 *M* potassium phosphate buffer (pH 7.2), 470 m*M* L-ornithine, 9 m*M* α-ketoglutaric acid, and 1.1 m*M* pyridoxal phosphate, and incubated for 20 minutes with shaking *(11).* The reaction was terminated with 0.5 mL of 10% trichloroacetic acid. After 20 min at room temperature, the samples were centrifuged to obtain a protein-free supernatant. To 0.5 mL of the supernatant, 0.5 mL of a 0.5% solution of δ-aminobenzaldehyde in absolute ethanol were added, mixed vigorously, and centrifuged to remove particulate matter, before absorbance was determined at 443 nm. The molar extinction coefficient of 2.71×10^3 was used to calculate the amount of product, and activity was expressed as μmol/h/g tissue.

The femurs were freed of excess tissue, cleaned to the periosteal surface with cheese cloth, and dried in a freeze-dry system (Labconco, Kansas City, MO). The air-dried basal diets and dried bone samples were ashed in platinum crucibles at 450°C utilizing a lithium–boron fusion technique *(12).* Bone and dietary macrominerals and trace elements were determined by inductively coupled argon plasma–atomic emission *(13).* Standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD) #1572 Citrus Leaves and #1577B Bovine Liver were used as quality control materials in the analyses of minerals. Replicate analysis of Bovine Liver yielded μg/g values of 117 ± 3 calcium, 145 ± 1 copper, 182 ± 1 iron, 8933 ± 4 potassium, 610 ± 4 magnesium, 9.5 ± 0.1 manganese, 3.70 ± 0.02 molybdenum, 2148 ± 2 sodium, 10850 ± 2 phosphorus, and 118.3 ± 0.3 zinc compared to certified values of 116 ± 4, 160 ± 8, 184 ± 25, 9940 ± 20, 601 ± 28, 10.5 ± 1.7, 3.5 ± 0.3, 2420 ± 60, 11000 ± 300, and 127 ± 16, respectively.

Data were statistically compared by using the *t*-test *(14).* Differences between values were considered significant when $p < 0.05$.

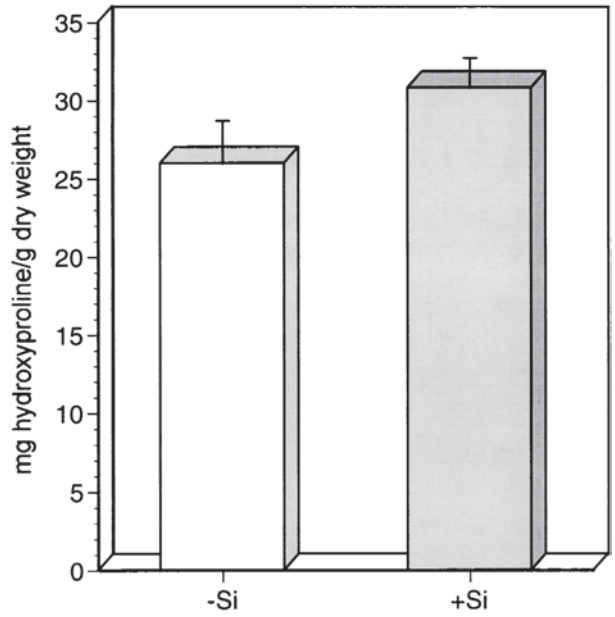

Fig. 1. Means are ± SD milligrams of hydroxyproline per gram of dry tibia of animals fed respective diets for 3 wk. Treatment was 0 Si or + Si (10 µg/g) supplemented to the diet (p=0.0009).

RESULTS

The growth of the rats as indicated by body weight in experiment 1 was not significantly affected by dietary silicon at wk 3, when the tibia bone was removed from 18 of the experimental animals. The total amount of hydroxyproline (mg/g dry wt) from the tibia was lower in the silicon-deficient animals than animals fed adequate silicon (*see* Fig. 1).

There was no significant difference in body weight or the wet weight of the implanted sponges removed from the rats at wk 5. The dry weight of sponges tended (p=0.06) to be lower in animals fed silicon-inadequate diets. However, the total amount of hydroxyproline was significantly lower (p<0.005) in sponges taken from silicon-deficient animals (5.70 ± 1.04 mg) than silicon-adequate (7.05 ± 1.05 mg) rats (*see* Fig. 2). The DPMs of ^{14}C-proline expressed per gram of dry tissue were significantly higher in extracts from sponges from silicon-deficient than silicon-adequate rats (*see* Fig. 3).

At 7 wk in experiment 2, dietary silicon did not affect the final weight, liver weight/body weight ratio, or red blood cell, white blood cell, or platelet counts (data not shown). However, hematocrit (46.1% vs 44.7%, p=0.05) and hemoglobin (14.53 vs 14.06 g/dL, p=0.04) were higher in silicon-

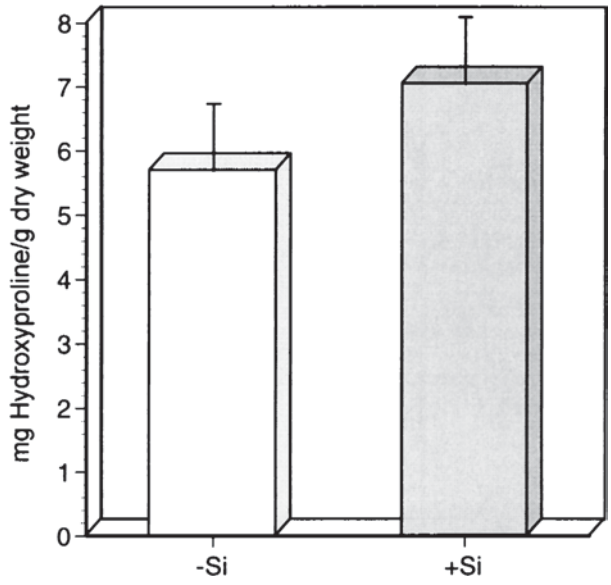

Fig. 2. Means ± SD are milligrams of hydroxyproline per gram of dry tissue collected in polyvinyl sponges over a 2-wk period. Treatment was 0 Si or + Si (10 µg/g) supplemented to the diet (p=0.0006).

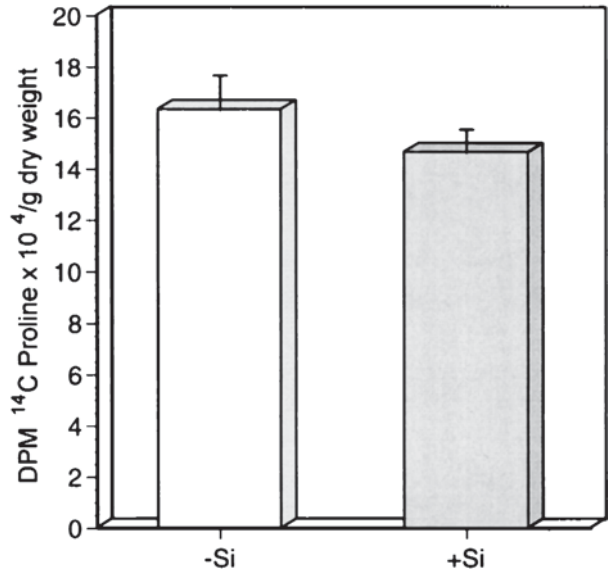

Fig. 3. Means ± SD are DPM ^{14}C-proline × 10^4 g dry tissue found in polyvinyl sponges 16 h after injection. Treatment was 0 Si or + Si (10 µg/g) supplemented to the diet (p=0.0003).

Table 1
Effect of Dietary Silicon on Selected Blood and Bone Variables

Variable	0 µg/g Si supplement	10 µg/g Si supplement	p value
Hematocrit (%)	46.1 ± 0.49[a]	44.7 ± 0.49	0.05
Hemoglobin (g/dL)	14.53 ± 0.17	14.06 ± 0.14	0.05
Eosinophils (%)	0.56 ± 0.06	0.79 ± 0.06	0.02
Femur Copper (µg)	1.44 ± 0.03	1.56 ± 0.03	0.0030
Femur Manganese (µg)	0.38 ± 0.04	0.63 ± 0.03	0.0001
Femur Molybdenum (µg)	2.89 ± 0.04	3.19 ± 0.04	0.0001
Femur Zinc (µg)	160 ± 3	160 ± 3	NS
Femur Calcium (µg)	175 ± 1	178 ± 1	NS
Femur Silicon (µg)	3.82 ± 0.77	5.04 ± 1.02	NS

[a] Mean ± SEM (n=12).

deprived than silicon-adequate rats (*see* Table 1). The silicon-inadequate animals also exhibited significantly less eosinophils expressed as a percentage of total white blood cells than animals fed adequate silicon (0.56% vs 0.78%, p=0.02). The activity of ornithine aminotransferase was lower in liver of silicon-deprived than silicon-adequate rats—50.7 versus 65.1 µmol/h/g tissue (*see* Fig. 4). Table 1 also shows that the concentrations of copper, manganese, and molybdenum in the femur were depressed by silicon deprivation. The concentration of silicon in bone was also in the expected direction when dietary silicon was inadequate. Dietary silicon did not affect femur calcium concentration.

DISCUSSION

Based on the femur mineral composition data in Table 1, which are similar to data obtained in previous silicon-deprivation studies, rats fed the low-silicon diet in the present experiment were silicon deficient. Thus, the findings in the present study show that silicon deprivation decreases the formation of collagen associated with wound healing, an enzyme involved

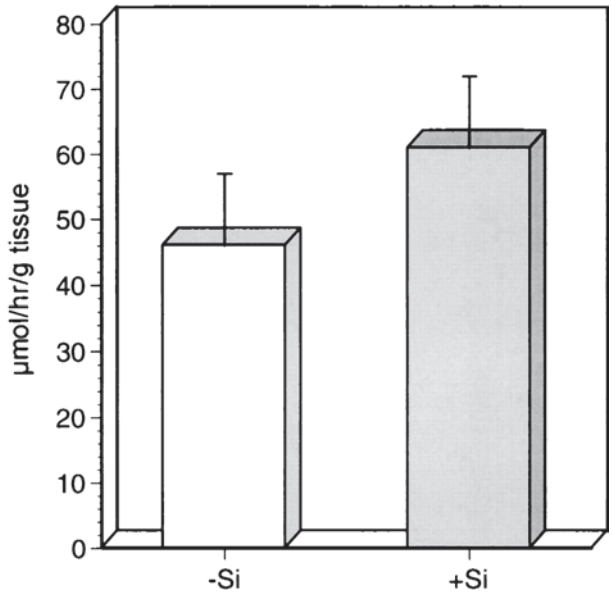

Fig. 4. Means ± SD are liver ornithine transaminase activity (µmol/h/g) wet liver weight. Treatment was 0 Si or +Si (10 µg/g) supplemented to the diet (p=0.003).

in collagen formation, and in eosinophils, that play an important role in wound healing. The depression in each of these variables could increase the length of time for wounds to heal, and the changes in sponge variables confirm this, that is, the decrease in sponge (wound) collagen and the increase incorporation of ^{14}C-proline into sponges placed in silicon-deficient rats most likely reflect delayed collagen formation in the wound-healing process. In other words, higher hydroxyproline (collagen) content with a lower ^{14}C-proline content (indication of an increased conversion to hydroxyproline) suggests that collagen formation or incorporation in the sponge was faster and greater in silicon-adequate rats than in the silicon-deficient rats. Moreover, this effect of silicon on collagen formation was induced by physiological, not pharmacological, amounts of silicon. The 10-µg Si/g diet used in the present study is lower than we found in rat diet composed of natural ingredients.

The successful healing of wounds requires the local synthesis of significant amounts of collagen. The amino acids that are the immediate precursors for collagen synthesis within wounds have not been specifically identified, but are likely to include the free amino acids contained in the extracellular wound fluid. Albina et al. *(15)* showed that this fluid is rich in proline and its metabolic precursors—ornithine, glutamate, and glutamine—and that ornithine contributes to the synthesis of protein-bound

proline in wounds by increasing the local extracellular pool of free proline. The decreased activity of liver ornithine aminotransferase, which converts ornithine to proline, in silicon-deficient animals indicates that silicon adequacy is also important for the activity of this enzyme in peripheral tissues, where it apparently is critical in promoting wound healing.

Song et al. *(16)* suggested that eosinophils play an important role in the organizational aspects of wound healing. Human eosinophils stimulate matrix production in dermal fibroblasts *(17).* Eosinophils also deliver cytokines involved in wound-healing process. Thus, our eosinophil findings are consistent with silicon being important for wound healing. Moreover, the dispersion of the collagenous fibers in areas around wounds that occurs before the rise in eosinophil numbers apparently can be affected by the sulfated glycosaminoglycans in the wound area *(18).* In addition to affecting eosinophil numbers, silicon nutriture can affect the glycosaminoglycan content of tissue. This was demonstrated by Carlisle *(19),* who found that silicon deprivation in chicks depressed the glycosaminoglycan content in connective tissue. Silicon also is consistently found as a component of some sulfated glycosaminoglycans.

Although not determined in the present study, a finding in another study by us *(20)* suggests that silicon could exert an influence on wound healing through still another mechanism. Hoffman et al. *(21)* reported findings indicating that T-lymphocytes at the wound site are crucial for the healing process. We found that when normal amounts of arginine were fed, silicon deprivation decreased the proliferation of splenic lymphocytes stimulated by concanavalin A *(20).* This effect indicates that silicon is needed for optimal proliferation of T-lymphocytes in the wound-healing process.

The present study also supports the hypothesis that silicon is important in bone formation through an effect on collagen formation or organic matrix composition. The finding that silicon deprivation decreased the collagen content in bone of 3-wk-old rats is consistent with results obtained in other studies. For example, Carlisle *(6)* reported that tibiae of silicon-deficient chicks had depressed contents of articular cartilage, water, hexosamine, and collagen. We found decreased bone collagen in rats fed a silicon-deficient diet for 9 wk *(22)*. We have consistently found that silicon deficiency decreases the copper concentration in bone *(9);* this was also found in the present study. Copper is needed for the crosslinking of collagen in bone. Thus, through an effect on copper, silicon might be affecting organic matrix composition or function and, ultimately, bone formation. Further support for silicon being important in collagen formation in bone is the finding of Carlisle *(23)* that maximal prolyl hydroxylase activity in cultured chick embryo frontal bones was dependent on silicon.

Interestingly, although collagen content in bone was decreased by silicon deprivation, the calcium content of bone was not significantly affected by dietary silicon in the present experiment. This is in contrast to findings obtained in other experiments by us *(9).* The difference, however,

probably was related to the age of the animals or the length of time the animals were maintained on the silicon-deficient diet. In previous experiments, weanling rats were fed the silicon-deficient diet for 8 or 9 wk before bones were obtained for analysis; in the present experiment, weanling rats were fed the silicon-deficient diets for only 3 wk.

Although the essentiality of silicon was suggested almost 30 yr ago, the nutrition community has generally ignored this element. Thus, the functional role of silicon in metabolism remains undefined and the practical nutritional importance of silicon has not been determined. As a result, the minimal silicon requirement for animals or humans has not been ascertained. A major criticism of silicon nutrition research has been the high amounts of silicon supplemented to diets of silicon-adequate controls. The present study shows that physiological amounts of silicon in the diet of adequate controls prevent undesirable effects induced by a diet containing low amounts of silicon. The amount of silicon in the diet of silicon-supplemented rats was actually much lower than some commercial rat chows; these often contain silicon near 1 mg/g. The physiological amounts of dietary silicon, compared to inadequate silicon, in the present experiment increased the activity of an enzyme necessary for collagen formation, enhanced collagen deposition in surgically implanted sponges, and increased circulating eosinophils. In a previous study, lymphocyte proliferation in response to mitogen was enhanced. These effects indicate that silicon is performing a critical and essential role, not a pharmacological role, in wound healing. Findings in the present study also show that silicon is involved in bone formation. Thus, silicon most likely is an element of concern in human and animal nutrition.

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